

CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

ETHEPHON

Chemical Code # 1626, Tolerance # 300
SB # 261

July 29, 1986
Revised: 6/10/87, 2/9/90, 9/20/91, 11/24/97

I. DATA GAP STATUS

| | |
|------------------------|--------------------------------------|
| Chronic toxicity, rat: | No data gap, no adverse effect |
| Chronic toxicity, dog: | No data gap, no adverse effect |
| Oncogenicity, rat: | No data gap no adverse effect |
| Oncogenicity, mouse: | No data gap, no adverse effect |
| Reproduction, rat: | No data gap, no adverse effect |
| Teratology, rat: | No data gap, no adverse effect |
| Teratology, rabbit: | No data gap, no adverse effect |
| Gene mutation: | No data gap, possible adverse effect |
| Chromosome effects: | No data gap, possible adverse effect |
| DNA damage: | No data gap, possible adverse effect |
| Neurotoxicity: | No data gap, no adverse effect |

Toxicology one-liners are attached.

All record numbers through 154981 and 987990 were examined.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: T971124

Revised by J. Kishiyama and S. Morris, 9/20/91; J Gee, 11/24/97

II. TOXICOLOGY SUMMARY

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

** 300-091 074763, "Lifetime Dietary Combined Chronic Toxicity and Oncogenicity Study with Ethephon in Albino Rats", (Bushy Run Research Center, Laboratory Project I.D. 51-501, May 16, 1989). Ethephon from various lots with purity ranging from 70.6% to 72.1% was administered in the feed at concentrations of 0, 300, 3000, 10000, or 30000 ppm to groups of 30 and 50 Sprague-Dawley CD® rats/sex/treatment rate for 97 (males) and 104 (females) weeks. No adverse effect. Systemic NOEL = 3000 ppm (Dose-related decrease in urine pH at ≥ 10000 ppm; decreased body weight gain at 30000 ppm). ChE NOEL = 300 ppm (plasma and RBC cholinesterase inhibition at ≥ 3000 ppm). Ethephon was not oncogenic in this study. ACCEPTABLE. (Kishiyama & Silva, 11/21/89).

CHRONIC TOXICITY, RAT

300-064 037712, "Two-year Dietary Administration Rats, Ethrel Final Report", (Hazleton, Report No. 141-205, 6/6/72). Ethephon 39.5%; fed to 60/sex/control group at 0 and 30/sex/group at 100, 300 or 1000/12,500 ppm over two years; NOEL: 100 ppm (ChE). Sacrifices of 10/sex/control and 5/sex/test group at 6 and 12 months. High dose at 1000 ppm, weeks 0-31, at 7500 ppm, weeks 32-36, at 10,000 ppm, weeks 37-41 and at 12,500 ppm, weeks 42-103 - no explanation. Clinical observations suggest intercurrent respiratory disease. No chronic effect related to test article; UNACCEPTABLE (incomplete description of test article, no justification of doses and why changed high dose 4 times, no analysis of diet, historical control data, or individual weights, no food intake, time to death, histopathology on all survivors at termination, inadequate number of animals at start, poor accountability of test animals. (Gee, 5/8/86).

EPA 1-liner: SUPPLEMENTARY. Sys NOEL = 100 ppm, ChE NOEL = 100 ppm.

300-018 987986 (1971, Hazleton) Pfeifer, 7/11/85. Six-month progress report for 300-064 037712.

300-003 046983. This document contains a brief summary of the six month progress report of a two year dietary study (probably the study at 300-064 037712) in rat using exposure levels of 100, 300, or 1000 ppm. No adverse effect was reported. No worksheet was done (S. Morris, 8/8/91).

300-064 037713, "104-Week Chronic Toxicity Study in Rats, Ethrel", (Hazleton, Report No. 141-263, 8/4/78). Ethephon source A 75.6%, source B 73.6%; 55/sex/group were fed 0, 30, 300 or 3000 ppm in the diet for two years from source A and a separate group of 30/sex from source B were fed 300 ppm; NOEL: 30 ppm (ChE); 3000 ppm (histopath); No onco or chronic effect attributable to the test article. UNACCEPTABLE. Need justification of doses, composition of other 25% of test article; analysis of diet over test period; husbandry conditions, individual clinical observations; individual body weights and food consumption. Hematology and clinical chemistry at week 13, 26, 53, 78 and 104 on 5/sex/group, same animals where possible. Plasma, rbc and brain cholinesterase were measured. Bone marrow cellularity varied but was comparable between control and test groups. Upgradeable. (Gee, 5/8/86).

300-067 037716,
300-068 037722, Supplemental to 300-064 037713.
EPA 1-liner: MINIMUM. ChE NOEL = 30 ppm; histopathology NOEL = 3000 ppm; sys
NOEL (body weight) = 300 ppm.

300-052 027146. This document contains a summary of 300-064 037713. No
worksheet was done (S. Morris, 8/7/91).

300-039 033587, "Long Term Toxicity and Carcinogenicity of Ethephon Formulation of 75%
Technical Ethephon to Rats", (No date or lab given). Very brief summary with unspecified
number of animals given up to 3000 ppm in the diet with no reported adverse effects. (Pfeifer,
7/5/85).

300-015 047068. This document contains a brief summary of "long term" study (probably the
study at 300-064 037713) in rat at "levels up to 3000 ppm." No adverse effect was reported.
No worksheet was done (S. Morris, 8/7/91).

CHRONIC TOXICITY, DOG

** 300-090 074653, "One Year Oral Toxicity in Dogs", (Hazleton Laboratories America Inc.,
HLA study no. 400-722, 5/30/89). Ethephon®, purity 71.4%, administered in the feed at
concentrations of 0, 100, 300, 1000, or 2000 ppm and fed to 5 Beagle dog/sex/group for 1 year.
No adverse effect. Spleen weight is reduced for the high dose groups, NOEL = 1000 ppm.
NOAEL \geq 2000 ppm (HTD). ACCEPTABLE. (Kishiyama & Silva, 11/21/89).

300-061 037585, "Two-year Dietary Feeding - Dogs Ethrel (39.5% AI)", (Hazleton, Report No.
141-206, 5/31/72). Ethephon, 39.5% Ethrel; 4/sex/group were fed 0, 50, 75, 100 or
250/7500/5000/6000 ppm in the diet over two years with 50 ppm being comparable to 1.5-3
mg/kg bw/day; NOEL: cannot be determined for chronic effects because the 100 and 75 ppm
groups were terminated after 16 weeks; NOEL < 50 ppm (ChE); decreased cellularity of bone
marrow and thickening of the duodenal wall at high dose; UNACCEPTABLE. Not Upgradeable.
Deficiencies: Problem with dose selection resulting in substantial changes over the 2 years;
inadequate histopathology in terms of tissues taken and findings reported; poor randomization at
start in terms of clinical chemistry, especially cholinesterase; justification for using test article at
39.5% - need description of remainder of material; no analysis of diet; no age of dogs is given;
no individual clinical observations. (Gee, 5/8/86).

EPA 1-liner: SUPPLEMENTARY. ChE NOEL < 50 ppm; sys NOEL (body weight) 50ppm.

300-017 987987. (1971, Hazleton). One year interim report for 300-061 037585. (Pfeifer,
7/8/85).

399-062 037586, "104-Week Dietary Administration-Dogs, Ethrel (Formulated - 39.5% AI) Final
Report", (Hazleton, Project No. 141-219, 9/28/72). Ethephon 39.5%, lot 68-250 (Ethrel
formulation); 4/sex/group were fed 0, 25, or 10/2500 ppm for two years; NOEL = 25 ppm; < 25
ppm for ChE; decreased femoral marrow cellularity (in females) and thickening of the duodenal
wall at the high dose; UNACCEPTABLE. Not upgradeable. Deficiencies: No justification of
dose and why the low dose was drastically increased well into the study, inadequate description
of the test article and the composition of the remaining 60%, no diet analysis over 2 years for
content of active ingredient, insufficient number of animals at termination (3), no individual
clinical observations. The low dose of 10 ppm was increased to 2500 at week 30. Hematology
at weeks 4, 13, 26, 52, 78, and 103. Clinical chemistry at the same intervals. One per sex per

group was sacrificed at 52 weeks. The report discusses the finding in the bone marrow of the femur in relation to the normal marrow found in the ribs and the negative peripheral blood differences suggesting that there may have been a problem in preparing the samples as well as questioning the significance. This finding was also reported in 037585 above. The finding of the thickening (hypertrophy) of the duodenal wall at 2500 ppm was also reported by Hazleton at 6000 ppm -see above. (Gee, 5/8/86).

EPA 1-liner: SUPPLEMENTARY. NOEL (sys) = 25 ppm; ChE NOEL < 25 ppm.

300-063 037711, "Two-year Dietary Study in Dogs - Ethrel Final Report", (Hazleton, Report No. 141-260, 11/17/77). Ethephon, (source A: 75.6%, B: 73.6% purity); 6/sex/group were fed 0, 30, 300 (A and B) or 3000/2000/1000/1500 ppm for two years; NOEL: 30 ppm (duodenal wall hypertrophy); cholinesterase inhibition at all doses; GI smooth muscle hypertrophy in 300 and 1500 ppm, source "A", but not source "B"; UNACCEPTABLE, possibly upgradeable.

Deficiencies: no justification of dose selection and why level was changed; needs complete description of test article; needs complete histopathology for all tissues preserved; no analysis of diet; age of dogs at start is not given. (Gee, 5/8/86)

EPA 1-liner: MINIMUM. RBC ChE NOEL = 30 ppm; Histopath. NOEL = 300 ppm.

300-052 027145. This document contains a summary of 300-063 037711. No worksheet was done (S. Morris, 8/7/91).

Two earlier chronic studies in dogs (see above) reported decreased cellularity in femoral bone marrow. One of them discounted this finding based on normal rib marrow and other hematological parameters. This report, 037711, indicates no remarkable findings. Taken all together, this effect may have been due to preparation of the marrow for examination as suggested in 037586. The other finding, however, of hypertrophy of the smooth muscle is supported by this study as well as the two earlier ones. (Gee, 5/8/86).

Conclusion: Based upon the latest study, performed according to FIFRA Guidelines, there were no adverse effects observed in beagle dogs, due to ethephon, over the required 1 year exposure period. There were questionable and inconsistent findings in the the bone marrow and certain hematological parameters, however when the study was repeated (074653) according to the FIFRA Guidelines, these phenomena were not observed. (Silva, 12/89).

ONCOGENICITY, RAT

See the combined chronic toxicity and oncogenicity study (CDPR 300-091 074763) above. Ethephon gave no indication of oncogenicity.

ONCOGENICITY, MOUSE

**** 300-087 072846**, "Lifetime Dietary Oncogenicity Study with Ethephon in Albino Mice", (Bushy Run Research Center, Project I.D. 51-502, 11/14/88). Ethephon, from various lots with purity ranging from 70.6% - 72.1%, administered in the feed at concentrations of 0, 100, 1000, or 10000 ppm to groups of 20 and 50 CD®-1 albino mice/sex/group for 52 and 78 weeks, respectively. No adverse effects. NOEL = 1000 ppm (decreased body weight and body weight gain in females; decreased urine pH in males). ChE NOEL = 100 ppm (significant plasma and erythrocyte inhibition was achieved at \geq 1000 ppm). NOAEL = 10000 ppm (HTD). An oncogenic

effect was not observed with ethephon. ACCEPTABLE. (Kishiyama & Silva, 11/22/89).

300-069 037717, "78-Week Oncogenic Evaluation in Swiss Albino Mice", (1981 and 8/85 for amended report, Food and Drug Research Labs, Report No. 5754). Ethephon, 75%; 85/sex/group were fed 0, 30, 300, or 1000 ppm in the diet, 78 weeks; NOEL: 30 ppm (ChE); no compound-related chronic or onco effect could be identified; UNACCEPTABLE, possibly upgradeable. Deficiencies: husbandry problems with 7 mis-sexed, 18 found dead in 1st fourteen weeks with no explanation, mite infestation, wet feeders, parasites; no description of the other 25% of the test article; no analysis of diet during test period; no justification of dose selection and no evidence MTD was reached for other than ChE depression (40-60% for plasma, 20-50% for rbc); no clinical observations or individual body weights; poor randomization with group 3 females being statistically heavier at start of study. (Gee, 5/9/86).

300-071 037718

300-070 037719

300-072 037720

300-073 037721, Supplemental to 300-069 037717.

EPA 1-liner: SUPPLEMENTARY as a cholinesterase study; invalid as an onco study based on inconsistencies in the histopathology data -- the report was amended and submitted as 037717-21 with correction of the discrepancies but there is no updated EPA 1-liner.

300-065 037714

300-066 037715. Original report of 300-071 037717 to 300-073 037721, reviewed by Gee, 5/9/86.

300-052 027156. This document contains a summary of 300-069 037717. No worksheet was done (S. Morris, 8/7/91).

REPRODUCTION, RAT

** 300-094 088512, T. L. Neeper-Bradley and R. W. Tyl, "Two-Generation Reproduction Study in CD® Albino Rats Exposed to Ethephon by Dietary Inclusion", Bushy Run Research Center, Laboratory Project ID 51-539, 5/17/90. A 2 generation (F0, F1B), 2 litter / generation (F1A, F1B, F2A, F2B) reproduction study was conducted in which Ethephon (Base A-250, approximately 71% stated purity) was administered in the feed at 0, 300, 3000, or 30,000 ppm to 28 parental rats/sex/dose/generation for a 10 week pre-breeding period, mating, gestation, and lactation. Twenty-eight pups/sex/dose were chosen from F1B litters for the second generation. Significant treatment-related effects in both sexes at 30,000 ppm were decreases in adult body weight gain, birth weights, and pup body weight gain (NOEL = 3000 ppm). No adverse effect was indicated. The study was ACCEPTABLE (J. Kishiyama and S. Morris, 8/5/91).

300-059 037582, "Three-generation Reproduction Study - Rats, Experimental Ethrel Final Report (39.5% AI)", (Hazleton, Report No. 141-214, 4/11/72). Ethephon, 39.5% Lot 68-250 ("experimental" Ethrel); 10 males and 20 females per group were fed 0, 200, 750 or 1500 ppm, three generations, two litters; NOEL: not established - > 1500 ppm; UNACCEPTABLE. Deficiencies: Incomplete (missing info - no individual data; body weight and food consumption for 3 of 9 weeks only, no justification of dose levels with no evidence of MTD from toxicity; no necropsy or histopathology information at all; inadequate description of test article, no age of animals. Report states "small pups" in all groups, all generations. Evidence of respiratory

disease in colony. (Gee, 5/6/86).

EPA 1-liner: GUIDELINE. Reproductive NOEL >1500 ppm.

300-017 987990 (Hazleton) Pfeifer 7/8/85 Summary of 300-059 037582

300-052 027147. This document contains a summary of 300-059 037582. No worksheet was done (S. Morris, 7/8/91).

300-014 047070. This document contains a brief summary of a three generation study (probably the study at 300-059 037582) in rats in which "dosage up to 1500 ppm" produced no compound-related effects. No worksheet was done (S. Morris, 8/8/91).

TERATOLOGY, RAT

300-089 074067, "Teratology Study with Ethephon Technical-Base 250 in Rats", (Hazleton Laboratories America Inc., Lab Project I.D. No. HLA 6224-125, 4/6/89). Ethephon technical, purity 71.7%, administered by gavage at concentrations of 0, 125, 250 or 500 mg/kg/day to 25 Crl:CD® (SD)Br female rats/group on day 6 thru 15 of gestation. No evidence of teratogenicity reported at dose levels in this study. No adverse effects. Maternal NOEL and embryo/fetal NOEL > 500 mg/kg. NOT ACCEPTABLE (no effects were observed at any dose level). (Kishiyama & Silva, 11/22/89).

300-059 037583, "Teratology Study in Rats - Ethrel (Ethephon)", (IRDC, Report No. 369-042, 11/18/80). Ethephon technical (solid), no purity stated ; 25/group were given 0, 200, 600, or 1800 mg/kg/day, 6-19, by oral gavage. NOEL: 600 mg/kg maternal toxicity, at 1800 mg/kg, 14/25 died. No indication of a teratogenic effect at any dose is reported. UNACCEPTABLE but upgradeable. Major deficiencies: no dosing analysis, no food consumption; only 1/3 were subjected to visceral examination instead of 1/2. Despite the high mortality at the high dose, 130 viable fetuses were available for examination so some estimate of fetotox-dev. toxicity could be made. The report does not indicate a teratogenic effect at 1800 mg/kg. (Gee, 5/7/86).

EPA 1-liner: MINIMUM. Teratogenic NOEL >600 mg/kg; poor survival at 1800 precludes usefulness for teratogenic effects. Maternal NOEL = 600 mg/kg (necrotic hepatitis, death. Fetotoxic NOEL >1800 mg/kg (HDT).

300-039 033586. Summary of 300-059 037583 (No date or lab given). (Pfeifer, 7/5/85).

300-079 051085. This document contains individual clinical observations for 300-059 037583.

300-079 051086. This document contains a description of the test article for 300-059 037583.

300-052 027154. This document contains a summary of an IRDC study (11/11/80, probably the study at 300-059 037583) that indicated no adverse effects at 200 or 600 mg/kg/day in a rat teratology study. No worksheet was done (S. Morris, 8/7/91).

300-015 047066. This document contains a brief summary of a study (probably the study at 300-059 037583) in rats using 0, 200, 600, or 1800 mg/kg. No adverse effects were reported at ≤ 600 mg/kg. No worksheet was done (S. Morris, 8/7/91).

Conclusion: Despite the deficiencies, it is evident in study 300-059 037583 that an MTD has

been reached and there are no teratogenic effects due to ethephon. Study 300-089 074067 shows results consistent with that of 300-059 037583 with a similar level for maternal NOEL. Although neither study alone is adequate, when taken collectively, 300-089 074067 and 300-059 037583 are acceptable for filling the rat teratology data gap. M. Silva, 2/9/90.

TERATOLOGY, RABBIT

** 300-098 087118, S. M. Henwood, "Teratology Study with Ethephon Technical - Base 250 in Rabbits", Hazleton Laboratories America, HLA 6224-158, 6/27/90. Twenty-two, virgin female Hra:(NZW)SPF rabbits / dose were artificially-inseminated (gestation day 0); injected with human chorionic gonadotropin (100 USP units / kg); exposed to ethephon (technical - base 250, lot # 4022193, purity \approx 72%, water vehicle) by oral gavage at 0, 62.5, 125, or 250 mg/kg on gestation days 7 through 19; and sacrificed on day 29. Maternal effects were behavioral abnormalities, reduced body weights, stomach lesions, and lethality (17/22) at 250 mg/kg/day. Except for death accompanying maternal lethality, no treatment-related fetal effects were reported. No adverse effect was indicated (maternal NOEL = fetal NOEL = 125 mg/kg/day). The study was acceptable (J. Kishiyama and S. Morris, 7/26/91).

300-059 037584, "Teratology Study in Rabbits, Technical Ethephon, Final Report", (Hazleton Labs, Report No. 400-635, 4/17/81). Ethephon technical, Lot aa, solid, 90% purity - 100% assumed; 17 does/group were given 0, 50, 100 or 250 mg/kg by oral gavage, days 6 through 19; high dose = 48% mortality; NOEL: 50 mg/kg (maternal mortality); no evidence of dev. toxicity without maternal toxicity. UNACCEPTABLE. Major deficiencies: no analysis of dosing solution for content or stability. Live fetuses were 71, 82, 47 and 25 at 0, 50, 100 and 250 respectively. (Gee, 5/7/86).

EPA 1-liner: SUPPLEMENTARY. Teratogenic NOEL > 50 mg/kg/day. Litters at termination were insufficient to determine teratogenic effect at 100 and 150 (sic) mg/kg/day. Embryotoxic-fetotoxic NOEL = 50; Maternal tox NOEL = 100 mg/kg/day (body weight gain, food consumption, mortality.)

300-079 050993. Individual maternal clinical observations for 300-059 037584.

300-052 027153. This document contains a summary of 300-059 037584. No worksheet was done (S. Morris, 8/7/91).

300-018 987989, "Segment II - Teratology - Rabbits Experimental Ethrel - Final Report", (Hazleton Labs, 10/21/70). Ethephon, 39.5% was fed in the diet to 12/per group at 0, 200 or 1000 ppm on days 8 -16 of gestation. No adverse effect indicated. NOEL (Maternal and fetal) > 1000 ppm. UNACCEPTABLE (Half of animals were sacrificed before term and half delivered; skeletal data for two groups should not be combined; no maternal body weight or food consumption; only 2 doses with no justification and no indication of maternal toxicity; no analysis of diet; no historical data; no sexing of fetuses/pups. Administration in the diet is not the preferred route for teratology study.) This study was originally reviewed with a potential adverse effect (Pfeifer 7/10/85). Reexamination of the data revealed no teratogenic effect due to ethephon since implantation occurs before dosing. In this study, however, there were too few does to evaluate fetal death. Therefore the study remains UNACCEPTABLE and not upgradeable. (Gee 6/6/86).

EPA 1-liner: SUPPLEMENTARY. Teratogenic NOEL >1000 ppm (HDT)

300-059 037581. (1970, Hazleton Labs). Exact duplicate of 018 987989.

300-015 047071. This document contains a brief summary of a rabbit teratology study (probably the study at 300-018 987989) that used levels "up to 1000 ppm." No adverse effect was reported. No worksheet was done (S. Morris, 8/7/91).

300-003 987988. This document contains a brief summary of a rabbit teratology study (probably the study at 300-018 987989) that used dietary exposures of 100 or 1000 ppm on gestation days 8 to 16. Increased fetal deaths were reported at 1000 ppm but too little data were given to ascertain the possibility of an adverse effect. No worksheet was done (S. Morris, 8/8/91).

GENE MUTATION

Mammalian cells

**** 300-087 073139**, "Mutagenicity Test on Ethephon Base 250: CHO/HGPRT Forward Mutation Assay", (Hazleton Laboratories America Inc., HLA Study No. 10065-0 -435, 1/19/88). Ethephon (presumed purity = 72.3%, EPA Est #NC264-01, Ref. #10-LJH-44) exposure concentrations (12 plates/concentration for mutation frequency) at 0, 0.5, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, or 5.0 mg/ml were tested with CHO-K1-BH₄ Chinese Hamster ovary (CHO) cell line, with and without Aroclor 1254 rat liver (S9-mix) activation, for 4 hours. A similar trial followed with Ethephon concentrations at 0, 0.5, 1.0, 2.0, 2.2, 2.4, 2.6, 2.8, or 3.0 mg/ml. An increase in mutation frequency observed for the 2.4/2.5 mg/ml dose was not confirmed in a second trial, therefore, ethephon is not considered to induce gene mutations in cultured Chinese hamster ovary cells. **Acceptable.** (Kishiyama & Silva, 11/22/89).

300-058 037697, "CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay Ethephon/Base 250", (Pharmakon, PH 314-UC-003-83, 12/29/83). Ethephon, 75% purity in water (Ethephon/Base 250) CHO exposed for 5 hours with and without activation to 0, 500, 1500, 1750, 2000 or 2500 ug/ml; no increase in mutation frequency is reported; duplicate cultures; ACCEPTABLE, with 037699. Retest at 2000 and 2500 ug/ml only +S9. Initially reviewed as unacceptable due to lack of test article. This has been submitted in 079 051086. (Gee, 5/1/86 and 6/1/87).

No EPA 1-liner.

300-079 051086. This document contains a description of the test article for 300-058 037697.

300-058 037699, "CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay Ethephon Base 250", (Pharmakon Research Int'l, PH 314-UC-001-84, 6/14/84). Ethephon Base 250 lot #A-41213; CHO were exposed for five hours with and without activation to 0, 166, 333, 1666, 3333, or 5000 ug/ml; cytotoxic at 5000 (12-14% survival); no increased mutation frequency noted in two trials. ACCEPTABLE with 037697. Initially reviewed as unacceptable due to lack of description of test article. This has been submitted in 079 051086. (Gee, 5/1/86 and 6/1/87).

No EPA 1-liner.

Microbial systems

**** 300-087 073138**, " Mutagenicity Test on Ethephon Base 250: In Ames Salmonella/Microsome Reverse Mutation Assay", (Hazleton Laboratories America, HLA study no. 10065-0-401, 10/12/87). Ethephon, purity 72.3%, exposure concentrations at 0 (deionized water), 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, or 50.0 ul/plate using Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100, with and without Aroclor 1254 rat liver (S9-Mix)

activation, for 48-72 hours (3 plates/dose & 2 trials). **An increase in his+ revertant colonies was observed with TA1535 (with and without S9) in both initial and repeated assays. Acceptable.** (Kishiyama & Silva, 11/22/89).

300-058 037701, "Ames Salmonella typhimurium/Microsome Plate Ethrel", (Pharmakon, PH 301-UC-001-80, 6/18/80). Salmonella, five strains; ethephon liquid, no purity stated ; bacteria exposed with and without rat liver S-9 to 50, 166, 500, 1666 or 5000 ug/plate, in triplicate, one trial; no increase in reversion rate reported. UNACCEPTABLE. Need description of test article and how dilutions were made; no individual plate counts, lacks some positive controls for some strains; no repeat trial. (Gee 5/1/86).

EPA 1-liner: ACCEPTABLE. Negative for TA1537, 1538, 98 and 100. Marginal results with 1535 with and without activation.

300-052 027150. This document contains a summary of 300-058 037701. No worksheet was done (S. Morris, 8/7/91).

300-058 037702, "Eukaryotic Reverse Mutation Saccharomyces cerevisiae - Ethrel", (Pharmakon, PH 303-UC-001-80 and 80A, 7/5/80). Etethephon, no purity stated.; Saccharomyces cerevisiae D7 - ilv I-92/I-92 exposed to 0, 0.42, 1.4, 4.2, 14 or 42 mg/ml, trial one, or 15, 30 or 45 mg/ml, trial two; no activation, only one hour, 20 plates each conc.; increase in revertants/ 10^7 at 42 and 45. Incomplete (missing data), UNACCEPTABLE - no description of test article, no activation, no rationale for 1-hour exposure instead of, e.g., 3-16 hours. (Gee, 5/1/86).

No EPA 1-liner.

300-052 027149. This document contains a summary of 300-058 037702. No worksheet was done (S. Morris, 8/7/91).

Summary: Etethephon was negative for gene mutation in mammalian cells (CHO) (3 studies: 073138, 037697 & 037699) but positive for increasing revertants in Saccharomyces and Salmonella TA1535 in 073139 both with and without metabolic activation (marginal results with TA1535 in 037701, according to the EPA). A positive effect without activation was also found in increased mitotic crossing-over and mitotic gene conversion in the same organism while negative findings were reported for other genotoxicity tests. Taken altogether, the Saccharomyces and Salmonella may be especially sensitive to this chemical. Therefore, etethephon can be considered to have a mutagenic effect in bacteria and yeast. Silva, 12/89.

CHROMOSOME EFFECTS

** 300-087 073140, "Mutagenicity Test on Etethephon Base 250: In an In Vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese hamster Ovary (CHO) Cells", (Hazleton Laboratories America Inc., HLA Study No.10065-0-437, 2/18/88). Etethephon, purity 71.3% by weight, at exposure concentrations of 0 (McCoy's 5a medium), 753, 1000, 1510, or 2010 ug/ml without metabolic activation for 7.25 hours and at 0, (McCoy's 5a medium), 502, 1000, 1510, or 2010 ug/ml with Aroclor 1254-induced rat liver (S9-mix) for 2 hours to Chinese Hamster (CHO-WBL) ovary cells (duplicate cultures, 100 cells scored/culture). There was no significant increase in chromosome aberrations. ACCEPTABLE. (Kishiyama & Silva, 11/27/89).

300-059 037694, "Evaluation of Ethrel on Spermatogenesis of Mice, Using the Dominant Lethal Test, Final Report", (Affiliated Medical Enterprises, Inc., 1/7/72). Etethephon technical, no purity stated; 20 males given 0, 100 or 1000 mg/kg by oral gavage and mated 1:1 over 5 periods (6-9, 13-16, 22-25, 36-39, and 42-45 days). UNACCEPTABLE protocol. Deficiencies: No

description of test article; no individual data; low % pregnancies (30% to 74% over 5 breeding periods; no clinical observations and no evidence of toxicity in main study; no husbandry information; no concurrent positive control. (Gee, 5/1/86).

No EPA 1-liner.

300-059 037695, "Dominant Lethal Study (Ethephon-56375) - Male Rats", (Pharmakon, 4/11/79). Ethephon Lot# 56375, no purity stated; ten males were given 0, 250, 500 or 1000 mg/kg for five doses by oral gavage then mated 1:2 over eight weekly periods; no dose-related dominant lethal effect is reported. UNACCEPTABLE (no purity of test article; inadequate number of pregnant females per period). TEM as positive control. (Gee, 5/1/86).

EPA 1-liner: No grade. Negative.

300-039 033588. Very brief summary of 300-059 037695 (No date or lab given). (Pfeifer, 7/5/85).

300-052 027152. This document contains a summary of 300-059 037695. No worksheet was done (S. Morris, 8/7/91).

300-015 047069. This document contains a brief summary of a study (probably the study at 300-059 037695) that reported a dominant lethal effect was not detected in male rats exposed to 250, 500, or 1000 mg/kg. No worksheet was done (S. Morris, 8/8/91).

300-058 037696, "Genetic Toxicology Micronucleus Test (MNT) Ethephon - Mice", (Pharmakon, PH 309A-UC-001-81, 4/6/81). Ethephon technical, no purity stated; four/sex/group were given 200 mg/kg i.p. - once or twice and sacrifice at 30 or 48 hours after one dose, 48 or 72 hours after two doses; no increase in MN's reported. UNACCEPTABLE, upgradeable. Deficiencies: No PCE/NCE for each animal; no purity for test article. This study used CD-1 mice. The other two used BS-1 strain. Also, the toxicity seems greater in this report in terms of clinical observations. Since none of the three reports describes the purity or lot number of the ethephon, that aspect cannot be evaluated. Also, the MN/1000 is much lower in controls and treated alike. Controls: #037708, 23.75; #037707, 15.5 and #037696, 1.5 MN/1000 cells. (Gee, 5/1/86).

EPA 1-liner: ACCEPTABLE. Negative.

300-052 027157. This document contains a summary of 300-058 037696. No worksheet was done (S. Morris, 8/7/91).

300-058 037707, "Genetic Toxicology Micronucleus Test Ethrel - Mice", (Pharmakon, PH 309-UC-002-80, 8/27/80). Ethephon, no purity stated; four/sex/group given 0, 400 or 600 mg/kg i.p., twice and sacrificed at six hours, increase in micronuclei over controls is reported. UNACCEPTABLE (protocol). Deficiencies: no purity for test article; single time of sacrifice at 6 hours only; inadequate number of animals (should be 5/sex/group) especially important in view of the scattering among animals; no PCE/NCE or MI for cytotoxicity evaluation of marrow. MN/1000 PCE's was 15.5 (control), 27.9 (400 mg/kg) and 26.6 (600 mg/kg bw). (Gee, 5/1/86).

No EPA 1-liner.

300-058 037708, "Genetic Toxicology Micronucleus Test Ethrel - Mice", (Pharmakon, PH 309-UC-001-80, 8/27/80). Ethephon, no purity stated; four/sex/group were given 0, 400 or 800 mg/kg twice by i.p. injection and sacrificed at six hours after second dose; an increase in MN

seen at 400 mg. UNACCEPTABLE (protocol). Deficiencies: No purity of test article; single time of sacrifice following two dosings; insufficient number of animals, no comment on PCE/NCE or cytotoxicity noted to indicate an adequate dose was used. Dose selection was based on a preliminary study. Since there were no deaths at 1 gm/kg, a higher dose than 800 mg/kg could have been used. MN/PCE's: 23.75 (control), 42.125 (400 mg/kg) and 30.75 (800 mg/kg). (Gee, 5/1/86).

No EPA 1-liner.

Summary: Although only a single study is acceptable (others are flawed in design or report), when all are considered collectively, CDPR concludes there are sufficient data to determine that ethephon has a potential for clastogenic effect, at least in one strain (BDS-1) of mouse.

DNA DAMAGE

** 300-087 073141, "Mutagenicity Test on Ethephon: In Rat Hepatocyte Unscheduled DNA Synthesis Assay", (Hazleton Laboratories America Inc., HLA Study No. 10065-0-447, 2/17/88). Ethephon (purity 71.3%), in exposure concentrations of 0 (WME), 25, 50, 250, or 1,000 ug/ml in one trial and 0 (WME), 500, 1,000, or 2,000 ug/ml in a second trial was used on rat primary hepatocytes (19 hour exposure). The test was performed by autoradiography and 150 cells were scored from triplicate cover slips/concentration. There was no indication of Ethephon induced DNA damage to in vitro rat hepatocytes. ACCEPTABLE. (Kishiyama & Silva, 11/27/89).

** 300-058 037698, "Rat Hepatocyte Primary Culture/DNA Repair Test, Ethephon Base 250", (Pharmakon, PH 311-UC-002-84, 6/13/84). Ethephon lot A-41213, 75% purity in water; hepatocytes were exposed 18-20 hours to 0, 0.3, 1.0, 10, 33, 100, 333, 1000, 3333 or 10,000 ug/well in 2 mls medium; no evidence of UDS; cytotoxic at 3333 and 10,000. ACCEPTABLE. Table I in text on analytical data needs an explanation. Initially reviewed as unacceptable due to lack of description of test article. This has been submitted in 079 051086. (Gee, 5/1/86 and 6/2/87).

No EPA 1-liner.

300-079 051086. This document contains a description of the test article for 300-058 037698.

300-058 037700, "DNA Polymerase Deficient Assay; Escherichia coli Ethrel", (Pharmakon, PH 305-UC-001-80, 5/23/80). Escherichia coli strains W3110 and P3478; ethephon, no purity stated; bacteria were exposed with and without S-9 to 0, 0.1112, 1.112, 11.12, or 1112.0 mg/ml (20 ul without S-9, 50 ul with rat liver S-9) with no differential effect on growths. UNACCEPTABLE, upgradeable with active ingredient description. (Gee, 5/1/86).

EPA 1-liner: ACCEPTABLE. Negative for DNA damage.

300-052 027151. This document contains a summary of 300-058 037700. No worksheet was done (S. Morris, 8/7/91).

300-058 037703, "Mitotic Crossing-over Saccharomyces cerevisiae Ethrel", (Pharmakon, PH 302-UC-001-80, 7/15/80). Ethephon liquid, no purity stated; strain D7 heteroallelic diploid ade2-40/ade2-119; 0, 0.42, 1.4, 4.2, 14 or 42 mg/ml, one hour, no activation only, 40 plates, one trial; suggestion of an increase at 42 mg/ml in mitotic crossing-over-gene conversion. UNACCEPTABLE (protocol). Deficiencies: Use of DMSO as solvent; no activation system included; high concentration should have been higher from cytotoxicity in preliminary trial

especially since there is a suggestion of an effect; rationale for 1 hour treatment rather than usual 3-16 hours. Percent twin-sector colonies: Control - 0.05; 0.42 - 0.06; 1.4 - 0.05; 4.2 - 0.04; 14 - 0.04 and 42 - 0.16. (Gee, 5/5/86).

EPA 1-liner: UNACCEPTABLE. Negative for mitotic crossing over. Not tested with metabolic activation or past 50% survival rate.

300-052 027148. This document contains a summary of 300-058 037703. No worksheet was done (S. Morris, 8/7/91).

300-058 037704, "Mitotic Gene Conversion, Saccharomyces cerevisiae, Ethrel", (Pharmakon, PH 304-UC-001-80 and -80A, 1980). Ethephon, no purity stated; Strain D7 heteroallelic diploid trp5-12/trp5-27; incubated with 0, 0.42, 1.4, 4.2, 14 or 42 mg/ml for one hour without S-9 (trial 1) or with 0, 15, 30 or 45 mg/ml without S-9 for one hour trial 2), 30 plates each; an increase in revertants to trp independence was reported for 42 and 45 mg/ml with a suggestion of a dose response. UNACCEPTABLE (no activation). Trial 1: Control - 1.17 revertants/10⁵ cells, 14 - 1.88, 42 - 4.21. Trial 2: Control - 0.67, 30 - 1.09 and 45 - 2.19. (Gee, 5/5/86).

EPA 1-liner: ACCEPTABLE. Produced an increase in reverse mutation in strain D7 at 42 and 45 mg/ml -S9. [Not clear from EPA if also positive for mitotic gene conversion - seems to be confusion with study numbers.]

300-052 021758. This document contains a summary of 300-058 037704. No worksheet was done (S. Morris, 8/7/91).

Conclusion: Saccharomyces appears to be sensitive to DNA damage by ethephon, where E. Coli and rat hepatocytes are not. Although the study with rat hepatocytes is negative, there are sufficient data from other studies to support the possibility that ethephon induces DNA damage.

NEUROTOXICITY

300-018 987973, "Neurotoxicity Study of Formulated Ethrel and Technical Ethrel on Hens - Final Report", (Hazleton, Report No. 141-218, 12/30/70). Ethrel formulated, purity not stated; 10 per group were given 0, 500 or 1000 mg/kg/day of technical or formulated active ingredient but schedule and rationale are unclear. Insufficient information to evaluate; UNACCEPTABLE. No delayed neurotoxicity is reported but protocol and dosing are inadequate for test. (Pfeifer, 7/9/85).

300-060 037709. Exact duplicate of 300-018 987973 (1970, Hazleton).

300-052 027155. This document is a summary of 300-018 987973. No worksheet was done (S. Morris, 8/7/91).

300-015 047065. This document contains a brief summary of a neurotoxicity study (probably the study at 300-018 987973) in hen. No adverse effect was reported. No worksheet was done (S. Morris, 8/7/91).

300-003 987971. This document contains a brief summary of a neurotoxicity study (probably the study at 300-018 987973) in hen. No adverse effect was reported. No worksheet was done (S. Morris, 8/8/91).

** 300-060 037710, "42-Day Neurotoxicity Study with Ethephon: Base 250 in Mature White Leghorn Chickens", (Bio-Life Associates Report No. 83 DN 102, 12/15/83). Ethephon 70.75%;

30/treated group were given 3850 or 3160 mg/kg by gavage on day 0; survivors (approx. 20) were given 2370 mg/kg on day 21. Fifteen/vehicle and positive control. No evidence of delayed neurotoxicity. ACCEPTABLE. Justification for using hens 17-18 months old is in 300-079, Tab (Gee, 5/7/86 and 6/1/87; Patterson, 6/1/87).

300-079 051086. This document contains a description of the test article for 300-060 037710.

300-270 154980 "A 2-week range-finding toxicity study of orally administered ethephon technical grade base 250 in rats." (P. Beyrouthy, Study Director, Bio-Research Laboratories, Canada, Project ID 97453, April 28, 1997) Six/sex/group of Sprague-Dawley Crl:CD®(SD)BR rats were given Etethephon technical base 250, 72.4%, lot no. 4051511, by gavage for 14 days. Doses were 0 (deionized water), 100, 300, 600 or 1000 mg/kg, corrected for purity with dosing solutions analyzed weekly. Animals were examined twice daily. The FOB was performed prior to dosing, on days 2, 8 and 15. Plasma and whole blood cholinesterase activity were measured prior to dosing on days 2, 8 and 15 after the FOB evaluation. Mortality: 2 males and 4 females at 600 mg/kg and all males and 5 females at 1000 mg/kg died or were sacrificed prior to scheduled termination. Clinical signs at these doses included fur staining, skin pallor, abnormal breathing, respiratory sounds, dehydration, cold to touch, decreased activity, weak appearance and abdominal distension. Minimal effects were seen at the lower doses. A few effects were noted in the FOB portion such as respiratory sounds, pinpoint pupils and impaired gait. No significant differences were detected in red blood cell cholinesterase while plasma cholinesterase levels were decreased at 300 mg/kg and higher. Only gross pathology was performed. SUPPLEMENTARY STUDY to 300-271, 154981. No worksheet. (Gee, 11/20/97).

** 300-271 154981 "A 13-week study of the potential effects of orally administered ethephon, technical grade base 250 on behavior, neurochemistry and neuromorphology" (P. Beyrouthy, ClinTrials BioResearch Ltd., Canada, Project I.D. 97414, 4/28/97) Groups of 22/sex/dose Sprague-Dawley rats [Crl:CD®(SD)BR] were treated with 0 (deionized water), 75, 150 or 400-300 [reduced in week 10/11] mg/kg by gavage. Three males and 3 females died at the high dose before it was reduced. Body weights, food consumption, clinical signs, a FOB and motor activity were determined. Blood, plasma and brain cholinesterase activity were recorded. Positive control data were submitted for a new active ingredient, cyclanilide [52093-081, -082 and -083]. Six per sex from the control and high dose groups were submitted to histopathology of the nervous system. Erythrocyte and plasma cholinesterase levels were depressed at all doses but brain cholinesterase was not affected. ChE NOEL < 75 mg/kg. Systemic NOEL = 150 mg/kg (mortality, clinical signs). No behavioral changes were observed and no treatment-related neuropathological lesions were found. ACCEPTABLE. (Gee, 11/24/97).